DOCUMENTED CHROMOSOME NUMBERS 2003: 1. CHROMOSOME NUMBER OF *TOXICOSCORDION NUTTALLII* (LILIALES: MELANTHIACEAE) AND CLARIFICATION OF THE GENUS

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ABSTRACT

The mitotic chromosome count for *Toxicoscordion nuttallii* ($2n = 22$), reported here, matches the number formerly documented for other species in the genus. A previous unvouchered report of $2n = 32$ for this species, therefore, likely represents a misidentification, and a base number of $x = 11$ is synapomorphic for *Toxicoscordion*. An updated overview of this recently resurrected segregate genus is given.

RESUMEN

El recuento de cromosomas mitóticos en *Toxicoscordion nuttallii* ($2n = 22$), citado aquí, concuerda con el número documentado anteriormente para otras especies del género. Un reporte previo, sin catalogar, de $2n = 32$ para esta especie, probablemente representa una identificación incorrecta y el número base de $x = 11$ es sinapomórfico para *Toxicoscordion*. Aquí se presenta una revisión de este género segregado recientemente resucitado.

Tribe Melanthieae (Liliales: Melanthiaceae) comprises seven genera (ca. 62–93 spp.) of predominately woodland and/or alpine perennial herbs occurring mainly in the temperate to Arctic zones of the Northern Hemisphere: *Amianthium* (1 sp.), *Anticlea* (9–11 spp.), *Schoenocaulon* (24 spp.), *Stenanthium* (2–3 spp.), *Toxicoscordion* (8 spp.), *Veratrum* s.l. (17–45 spp.), and *Zigadenus* s.s. (1 sp.). These generic circumscriptions (most novel) are supported by recent parsimony analyses of *trnL-F* (plastid) DNA and ITS (nuclear ribosomal) sequence data (Zomlefer et al. 2001, 2003). A significant consequence of the molecular studies was the reassessment of the traditional *Zigadenus* s.l., a poorly defined assemblage with a complex taxonomic history (summary in Zomlefer 1997). Although several segregates of *Zigadenus* have been described, contemporary treatments (e.g., Schwartz 2002) have typically accepted only the monotypic segregate *Amianthium* with the remaining ca. 19 species maintained in *Zigadenus* s.l. Based on these molecular data, however, *Zigadenus* s.l. is polyphyletic and forms five strongly supported clades that correlate with certain geographical distribution, morphological characters, and chromosome number.

One well-defined clade corresponds to the genus *Toxicoscordion*, originally described by Rydberg (1903), and recognized as a distinctive taxon by both Preece (1956) and Schwartz (1994) as *Zigadenus* sect. *Chitonia* (R.A. Salisbury) J.G. Baker. This *Zigadenus* segregate comprises ca. eight species (Table 1) restricted to the midwestern United States to western North America (Fig. 1A) and includes the well-known poisonous "death camas" plants of the rangelands such as *T. nuttallii*, *T. paniculatum*, and *T. venenosum* (see Marsh et al. 1915, 1926). The resurrection of *Toxicoscordion* required one new combination (*T. fontanum*), a species described within *Zigadenus* by Eastwood (1937), long after Rydberg (1903) had circumscribed the genus and had transferred the appropriate contemporary *Zigadenus* species. *Toxicoscordion* is defined by the morphological synapomorphies of conspicuously clawed tepals (especially the inner three) and one obovate gland per tepal (Fig.1B; Zomlefer et al. 2001; Zomlefer & Judd 2002). Although a discrete genus, specific and infraspecific limits are unclear, especially within the variable *T. micranthum*–*T. fremontii* species complexes and the intergrading *T. paniculatum*–*T. venenosum*. For example, in an isozyme study of these species (Schwartz 1994), the monophyly of *T. paniculatum* is suspect because the five sampled populations were separated from each other in UPGMA cluster analyses and distance Wagner trees. These results warrant further examination of the species complexes in the genus.

The chromosome number $2n = 22$ (or $n = 11$) has been reported for all species of *Toxicoscordion* (Table 1; Fig. 2) except for one anomalous report of $2n = 32$ for *T. nuttallii*. Since chromosome number is a significant and likely an invariable apomorphy for genera of tribe Melanthieae (e.g., Zomlefer and Smith 2002), 22 is the predicted mitotic count for all *Zigadenus* species now transferred to *Toxicoscordion*. This study challenges the earlier conflicting *T. nuttallii* report, thereby seeking to validate the diploid number of 22 as a consistent synapomorphy for the genus.

MATERIALS AND METHODS

Several bulbs of *Toxicoscordion nuttallii* were collected during April 2002 at two localities in south-central Texas by J. Spangler and T. Wendt (see Table 2) and transplanted to pots maintained at the Plant Biology Dept. Greenhouse Facility at the University of Georgia. Dividing root tip cells were prepared for examination with some modification of the general protocols outlined by Flory and Smith (1980) and Jones and Luchsinger (1986). Once the plants were well-established, actively growing root tips were harvested at 8:00 AM and treated with 0.2 % colchicine for 4 hours, rinsed in distilled water, and then fixed in Carnoy's solution (3 ethanol: 1 acetic acid) overnight or longer. Following this fixation, the roots were rinsed in distilled water and stored under refrigeration in 70% ethanol. The material was subsequently hydrolyzed in 1.0 N HCl at 53° C for 5 minutes, rinsed several times again with water, and treated with 45%

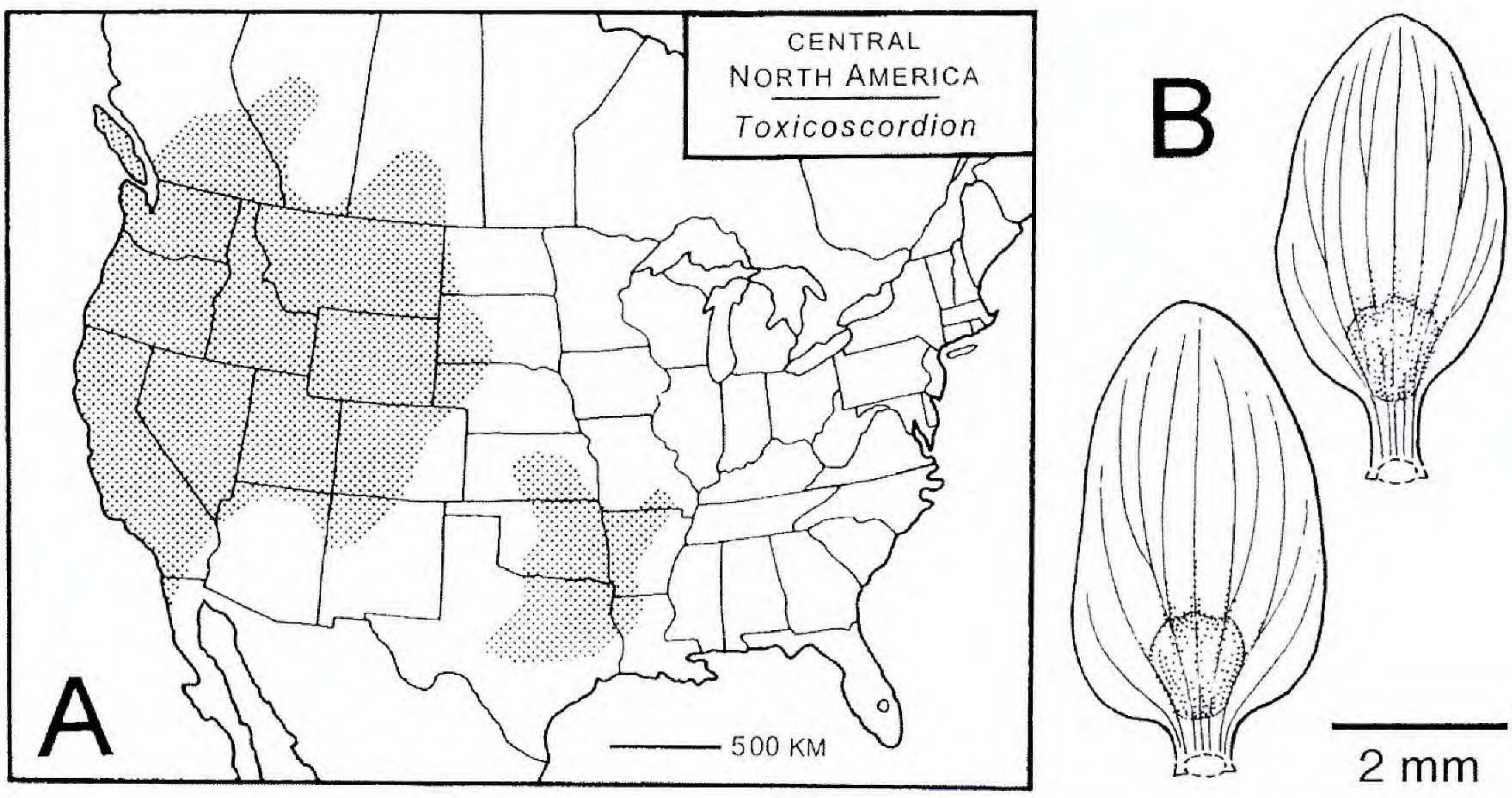

Fig. 1. Distinctive characteristics of *Toxicoscordion*. **A.** General distribution map of *Toxicoscordion* (data from Walsh 1940; Preece 1956; Schwartz 1994, 2002; M. H. MacRoberts pers. comm.). The midwestern disjunct range (Texas–Oklahoma–Kansas–Missouri–Arkansas–Louisiana) comprises the localities of *Toxicoscordion nuttallii*. **B.** Adaxial surface of an outer (left) and an inner (right) tepal from *T. nuttallii* (*Rollins 5334*, GH), showing the generic autapomorphies: claw plus a single obovate gland. Dashed ellipse = filament insertion. The claws of the inner tepals are typically more pronounced than those of the outer whorl.

glacial acetic acid for 10 minutes. The growing tips were then removed, macerated with a dissecting spatula on a glass microscope slide, and stained with 1% aceto-orcein. After application of a cover slip, the slide was gently heated with an alcohol lamp, placed between blotters, and subjected to additional pressure. Slides were mounted in euparal for future reference. Well-spread metaphase (and late prophase) chromosomes were traced under a Leica DMLB Research Microscope with a camera lucida attachment. Herbarium specimen vouchers (Table 2) are deposited at GA and TEX.

RESULTS AND DISCUSSION

The mitotic chromosome number of $2n = 22$ for *Toxicoscordion nuttallii* is confirmed with plants from populations in Hays and Williamson Counties, Texas (Fig. 2E, F; Table 2). A citation of $2n = 32$ for *T. nuttallii*, originally reported by Zakharieva and Makushenko (1969) and subsequently cited in various indices and floras (e.g., Fedorov 1969; Moore 1971, 1973; Churchill 1986; Yatskievych 1999), was based on an undescribed and unvouchered plant then growing at the Munich Botanical Garden (original source not cited). This "*Zigadenus*" species was likely an *Anticlea*, another *Zigadenus* segregate, but one characterized by $x = 8$ (Zomlefer & Judd 2002).

Chromosome number is a useful taxonomic character for genera within tribe Melanthieae, especially the synapomorphic $2n$ numbers of 20 for

TABLE 1. The species of *Toxicoscordion* and their mitotic (*2n*) and/or meiotic (*n*) chromosome numbers. The single citation for *T. nuttallii*, *2n* = 32, is inconsistent with reports for all other species in the genus.

Taxon	Chromosome Number n	Chromosome Number 2n	Secondary Citation(s) in Indices and Floras	Original Source(s)
T. brevibracteatus (M.E. Jones) R.R. Gates	11	—	Cave (1960); Fedorov (1969); Moore (1972, 1973); McNeal (1993)	Lewis (1959); Cave (1970)
T. exaltatum (Eastwood) A. Heller	11	—	McNeal (1993)	?
T. fontanum (Eastwood) Zomlefer & Judd	11	—	Moore (1972, 1973); McNeal (1993)	Preece (1956, as *Zigadenus venenosus* var. fontanus); Cave (1970)
T. fremontii (J. Torrey) Rydberg	11	—	Darlington & Wylie (1956); Fedorov (1969); Moore (1972, 1973); McNeal (1993)	Miller (1930); Preece (1956, as *Zigadenus fremontii* var. *fremontii*); Cave (1970, as *Z. fremontii* var. *fremontii* and var. *inezianus*)
T. micranthum (Eastwood) A. Heller	11	—	McNeal (1993)	Preece (1956, as *Zigadenus venenosus* var. *micranthus*)
T. nuttallii (A. Gray) Rydberg	—	32	Fedorov (1969); Moore (1971, 1973); Churchill (1986); Yatskievych (1999)	Zakharyeva & Makushenko (1969)
T. paniculatum (Nuttall) Rydberg	11	22	McNeal (1993)	Preece (1956)
T. venenosum (S. Watson) Rydberg	11	22	Moore (1972, 1973); Goldblatt (1981); Churchill (1986); McNeal (1993)	Preece (1956, as *Zigadenus venenosus* var. *gramineus* and var. *venenosus*); Cave (1970); Taylor & Taylor (1977, as *Z. venenosus* var. *gramineus*); Hartman & Crawford (1971, as *Z. venenosus* var. *gramineus*)

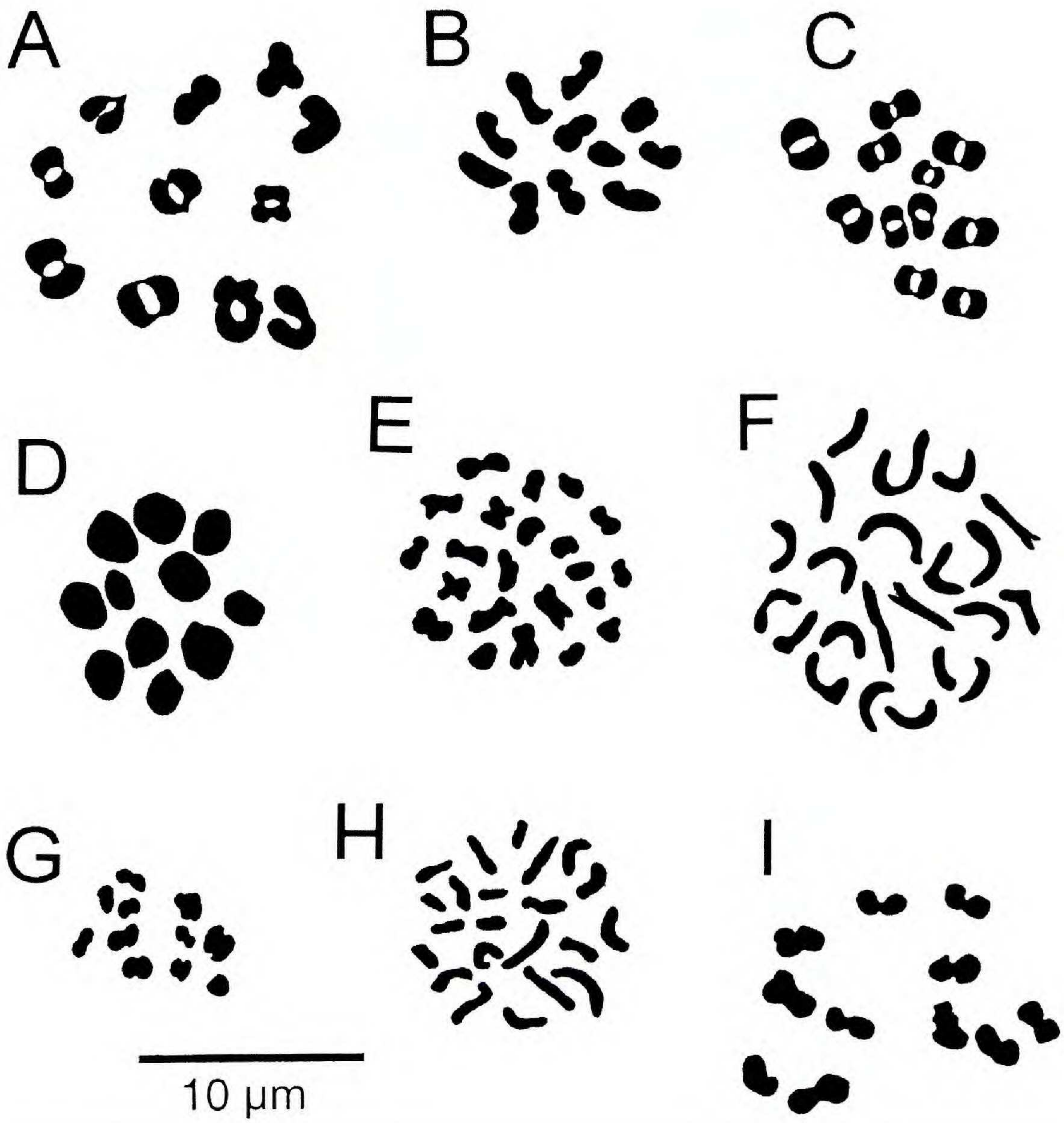

Fig 2. Summary illustration of previously documented meiotic (n = 11) and/or mitotic ($2n$ = 22) chromosomes of *Toxicoscordion* (A–D, G–I) and the new reports for *T. nuttallii* (E, F: $2n$ = 22). **A.** *T. brevibracteatus*, diakinesis (n = 11). **B.** *T. fontanum*, anaphase II (n = 11). **C.** *T. fremontii*, metaphase I (n = 11). **D.** *T. micranthum*, metaphase I (n = 11). **E.** *T. nuttallii* (*Wendt 7246*, TEX), metaphase ($2n$ = 22). **F.** *T. nuttallii* (*Spangler s.n.*, GA), late prophase ($2n$ = 22). **G.** *T. paniculatum*, metaphase ($2n$ = 22). **H.** *T. paniculatum*, metaphase I (n = 11). **I.** *T. venenosum*, metaphase I (n = 11). Figures A, B, and I modified from Cave (1970); Figs. C, D, G, and H modified from Preece (1956).

Stenanthium (Zomlefer & Judd 2002) and 22 for *Toxicoscordion*. A probable base chromosome number of $x = 8$ is often cited for the tribe (Sen 1975; Tamura 1995; Lowry et al. 1987; Zomlefer 1997), and multiples of this number are prevalent in other genera of the tribe: *Amianthium* ($2n$ = 32), *Anticlea* (including *Stenanthella*; $2n$ = 32), *Schoenocaulon* ($2n$ =16), and *Veratrum* (including *Melanthium*; $2n$ = 16, 32, 64, 80, 96). Due to the small size of the chromosomes

TABLE 2. Voucher specimens for the chromosome numbers of *Toxicoscordion nuttallii* reported in this study.

Taxon	Locality and collection number (voucher location)	Mitotic chromosome number (2n)
Toxicoscordion nuttallii	TEXAS. HAYS CO.: Between Driftwood and Dripping Springs, along FM 150, 3.7 mi N of FM 1826, 15 Apr 2002, *Wendt 7246* (TEX)	22
	TEXAS. WILLIAMSON CO.: Austin, 0.25 mi from 8521 Foxhound Tr., 3 Apr 2002, *Spangler s.n.* (GA)	22

(ca. 2.0-4.0 μm in length), however, the few detailed karyological studies (e.g., Lee 1985) lack the detail to infer mechanisms of chromosomal evolution, although these chromosome numbers indicate the prevalence of polyploidy and/or aneuploid variation of the prospective basic number. The validation of $2n$ = 22 for *T. nuttallii* strengthens support for the monophyly of *Toxicoscordion*, as well as the phylogenetic significance of chromosome numbers as generic synapomorphies for tribe Melanthieae.

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